OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Plant DNA DS Kit

M1130-00	1 x 96 preps
M1130-01	4 x 96 preps

September 2015

For research use only.Not intended for diagnostic testing.

Mag-Bind® Plant DNA DS Kit

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Introduction

The Mag-Bind® Plant DNA DS Kit allows rapid and reliable isolation of high-quality genomic DNA from difficult plant species and tissues. The lysis and binding buffers are specifically designed to minimize co-purification of polysaccharides and polyphenols. Up to ninety-six 50 mg samples of wet tissue (or 15 mg dry tissue) can be processed in less than one hour. The system combines Omega Bio-tek's E.Z.N.A.® buffer chemistry with the convenience of Mag-Bind® Particles to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. This kit is designed for manual or fully automated high throughput preparation of genomic, chloroplast, and mitochondrial DNA. Purified DNA is suitable for PCR, restriction digestion, Next Generation Sequencing, and hybridization applications. There are no organic extractions thereby reducing plastic waste and decreasing hands-on time to allow multiple samples to be processed in parallel.

New in this Edition:

RNA Binding Buffer has been replaced with RBB Buffer.

Kit Contents

Product Number	M1130-00	M1130-01
Preparations	1 x 96	4 x 96
CSPL Buffer	80 mL	2 x 150 mL
RBB Buffer	60 mL	240 mL
CSPW1 Buffer	39 mL	143 mL
CSPW2 Buffer	12 mL	44 mL
SPM Wash Buffer	36 mL	2 x 75 mL
Elution Buffer	60 mL	240 mL
CB Buffer	50 mL	200 mL
Proteinase K Solution	2.2 mL	8.8 mL
RNase A	1.5 mL	3.2 mL
Mag-Bind® Particles HDQ	2.2 mL	9 mL
Mag-Bind® Particles RQ	2.2 mL	9 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Plant DNA DS Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Store Mag-Bind® Particles HDQ, Mag-Bind® Particles RQ, and RNase A at 2-8˚C. All other materials should be stored at room temperature.

Preparing Reagents

1. Dilute CSPW1 Buffer with 100% ethanol as follows and store at room temperature.

CSPW1 Buffer	100% Ethanol to be Added
M1130-00	21 mL
M1130-01	77 mL

2. Dilute CSPW2 Buffer with 100% isopropanol as follows and store at room temperature.

CSPW2 Buffer	100% Isopropanol to be Added
M1130-00	48 mL
M1130-01	176 mL

3. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

SPM Wash Buffer	100% Ethanol to be Added
M1130-00	84 mL
M1130-01	175 mL

Mag-Bind® Plant DNA DS Kit - Protocol for DNA Isolation from Fresh or Frozen Specimens

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,000-5,000 x *g*
- Rotor adapter for 96-well deep-well plates
- Magnetic separation device for 96-well deep-well plates
- 96-well deep-well plates compatible with magnetic separation device(Recommended Alp Aqua Magnum EX (A000380))
- Incubators capable of 56°C and 65°C
- Vortexer
- Equipment for disrupting plant tissue (Geno/Grinder 2010 or MM300 Mixer Mill and tungsten carbide beads)
- 8- or 12-channel pipette
- Reagent reservoir
- Sealing film
- Sealed deep-well plate or capped microtube rack for sample disruption
- 100% ethanol
- 100% isopropanol
- Optional: 85% ethanol
- Optional: nuclease-free water

Before Starting

- Prepare CSPW1 Buffer, CSW2 Buffer, and SPM Wash Buffer according to the instructions in the Preparing Reagents section on Page 4
- Set an incubator to 56°C
- Heat Elution Buffer to 65°C

1. Grind 30–50 mg plant sample using a mechanical grinder such as Geno/Grinder.

   Note: To prepare samples in 96-well plate format, place samples in a sealed 96-well deep-well plate or capped microtube rack in the presence of one or two grinding beads. Process in the MM300 Mixture Mill or Geno/Grinder Mixture Mill following the manufacturer's instructions.

2. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each well. Vortex to mix thoroughly.

3. Incubate at 56°C for 30 minutes.

4. Centrifuge at 4,000 x *g* for 10 minutes.

5. Carefully transfer 500 µL cleared lysate to a new 96-well deep-well plate, making sure not to disturb the pellet or transfer any debris.

   Note: It is critical to leave the pellet undisturbed and avoid transferring debris as these can reduce yield.

6. Add 5 µL RNase A. Vortex to mix thoroughly.

7. Let sit at room temperature for 2 minutes.

8. Add 500 µL RBB Buffer and 20 µL Mag-Bind® Particles HDQ. Vortex to mix thoroughly.

9. Let sit at room temperature for 5 minutes. Vortex briefly every 90 seconds to resuspend magnetic beads.

   Note: If using a liquid handler with orbital shaker, continue to shake for the entire duration of this step.

10. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

12. Remove the plate from the magnetic separation device.

13. Add 500 µL CSPW1 Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles HDQ.

    Note: CSPW1 Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

16. Remove the plate from the magnetic separation device.

17. Add 500 µL CSPW2 Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles HDQ.

    Note: CSPW2 Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

18. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

20. Remove the plate from the magnetic separation device.

21. Add 500 µL SPM Wash Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles HDQ.

    Note: SPM Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

22. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

24. Repeat Steps 20-23 for a second SPM Wash Buffer wash step.

25. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles HDQ. Remove any residue liquid with a pipettor.

Alternative Ethanol Removal Step: Instead of performing Step 25, complete the step below.

- With the plate on the magnetic separation device, add 500 µL nuclease-free water and immediately aspirate (within 60 seconds).
- Continue to Step 26 below.

26. Remove the plate from the magnetic separation device.

27. Add 100-200 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles HDQ.

28. Incubate at 65°C for 5 minutes.

29. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

30. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate and store at -20°C.

    Note: If performing the secondary purification protocol (continue to next page), transfer the supernatant to a new 96-well plate with a capacity of 800 µL.

Optional Secondary Purification Protocol

The following steps can be performed if the samples contain any coloration or Elution Buffer is found to be viscous during pipetting. Performing an additional purification can help improve downstream performance.

31. Add 400 µL CB Buffer and 20µL Mag-Bind® Particles RQ to the sample. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles RQ.

32. Let sit at room temperature for 3 minutes.

33. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

34. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

35. Leave the plate on the magnetic separation device.

36. Add 400 µL 85% ethanol.

37. Let sit at room temperature for 30 seconds.

38. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

39. Repeat Steps 35-38 for a second ethanol wash step.

40. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles RQ. Remove any residue liquid with a pipettor.

Alternative Ethanol Removal: Instead of performing Step 40, complete the step below.

- With the plate on the magnetic separation device, add 400 µL nuclease-free water and immediately aspirate (within 60 seconds).
- Continue to Step 41.

41. Add 50-200 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles RQ.

42. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

43. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate.

44. Store at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yields	Incomplete disruption of starting material	For both fresh and frozen samples, make sure to grind samples completely.
	Poor lysis of tissue	Decrease amount of starting material. Increase lysis time to overnight.
	DNA lost during wash	Dilute SPM Wash Buffer by adding appropriate volume of ethanol prior to use (Page 4). If performing water wash, step proceeded for more than 60 seconds or plate was removed from magnetic separation device.
Problem	Cause	Solution
Problems in downstream applications	Salt carryover	SPM Wash Buffer must be at room temperature.
	Ethanol carryover	Dry the Mag-Bind® Particles HDQ completely before adding elution buffer. Perform Water Wash step instead of drying magnetic beads

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
SPM Wash Buffer (40 mL)	PS014
RNase A (5 mL)	AC118
96-well Microplate (500 µL) (5/pk)	EZ9604-01